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The Role and Function of Fc γ Receptors on Myeloid Cells

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A key determinant for the survival of an organism is the ability to recognize and respond to invading pathogens without damaging host tissues. This is largely accomplished by the concerted activity of the innate and adaptive branches of the immune system that efficiently eliminate invading pathogens and restore tissue homeostasis. An initial step in the generation of robust immune responses is the recognition of pathogens by host cells, triggering subsequent immune cell activation and induction of pro-inflammatory responses. This initial recognition is facilitated via pathogen-associated molecular patterns (PAMPs) that represent highly-conserved molecular structures uniquely found in bacterial, viral and fungal pathogens, but not in host tissues. Such structures include peptidoglycans, zymosan, lipopolysaccharides, flagellin, double-stranded and single-stranded RNA, and CpG-containing DNA (1). So far, an ever-increasing number of receptors with the capacity to sense and respond to these PAMPs have been identified and are broadly categorized into distinct receptor families: Toll-like receptors (TLR), RIG-1 (retinoic acid-inducible gene I)-like receptors (RLR), NOD-like receptors, and C-type lectin receptors (2–4). Following engagement, these pattern-recognition receptors trigger the activation of several inflammatory pathways essential to mediate robust anti-microbial activity and induce sustained immune responses. This central role in immunity for pathogen sensing by innate immune receptors is also reflected by the emergence of pattern-recognition receptor early in evolutionary history, as evidenced by the presence of highly-conserved gene orthologues in invertebrate species. Additionally, genetic analysis of human *TLR* and *NOD* genes provided evidence for strong positive selection pressure in human populations, and several non-synonymous polymorphisms influencing receptor activity have been associated with disease susceptibility (1).

Despite the key role for innate immune receptors in mediating pathogen recognition and responses by effector leukocytes, they present limited capacity to recognize pathogens of infinite diversity. Another key mechanism for recognition and clearance of non-self material is accomplished by IgG antibodies, which provide specific recognition of antigens of almost unlimited diversity. Indeed, through the diversification of their variable domains (VH and VL), antibodies have the capacity to specifically recognize diverse antigens, providing effective host protection during an immune response. Contrary to the antigen-binding Fab domain that exhibits astonishing variability, antibodies also comprise a relatively constant domain, the Fc domain. Recognition and binding of antibodies to the surface of the

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leukocytes is mediated through interactions of their Fc domains with specialized receptors, Fc receptors, expressed by several types of circulating and tissue-resident leukocytes (5). By directly linking molecules of the adaptive immunity with innate leukocytes, Fc receptors represent an important component that links both branches of immunity, enabling innate immune cells to specifically recognize and respond to antigens of unlimited diversity.

While traditionally termed the ‘constant domain/region’ of the antibody molecule, the Fc domain is, in fact, heterogeneous in both primary amino acid sequence (IgG subclass), and in the composition of the Fc-associated glycan (5–7). These two determinants regulate the structure and conformational flexibility of the IgG Fc domain and, in turn, determine interactions with various Type I and Type II Fc receptors (FcγR). Indeed, recent crystallographic studies support the existence of two main conformational states for the Fc domain: an “open” and a “closed” that are determined by the Fc-associated glycan structure; a highly conserved glycan site present in all human IgG subclasses and among many mammalian species (8, 9). In view of the two conformational states of the Fc domain, FcγRs can be categorized into type I and type II receptors, based on their capacity to interact with the “open” or the “closed” Fc domain conformation, respectively (8, 9). Engagement of type I and type II FcγR by the Fc domain is a tightly regulated process that is primarily determined by the conformational flexibility of the Fc domain and results in the induction of pleiotropic activities by effector leukocytes (5, 10).

IgG Fc domain heterogeneity and structural flexibility

The highly flexible structure of the Fc region is indicative of the unique structural organization of its different domains. In particular, the Fc region is composed of the two constant domains (CH2 and CH3) of the two heavy chains that form homodimers through tight association of the two CH3 domains at the C-terminal proximal region of the IgG as well as the presence of disulfide bonds in the CH2-proximal hinge region (11). This results in a characteristic horseshoe-like conformation, with the two CH2 domains forming a hydrophobic cleft, where the central *N*-linked glycan structure is localized. This Fc-associated glycan is conjugated to the amino acid backbone of the CH2 domain at position Asn297 and is required to maintain the Fc domain in a structural conformation permissive for interactions with FcγRs (12, 13)(Figure 1A). Indeed, genetic deletion of the Asn297 site or enzymatic removal of the Fc-associated glycan abrogates the capacity of the Fc domain to interact with FcγRs (14–17). In addition, an ever-increasing body of evidence strongly suggests a crucial role for the Fc-associated glycan in the regulation of Fc domain interactions with type I and type II FcγRs through modulation of the Fc domain flexibility (15, 16).

The Fc-associated glycan structure consists of a core heptasaccharide structure that is attached to the CH2 domains of each of the two heavy chains that comprise the Fc domain (12, 15)(Figure 1A). Previous analyses of the Fc glycan composition revealed substantial heterogeneity at steady-state conditions, due to the selective addition of fucose, galactose, *N*-acetylglucosamine and sialic acid residues to the core glycan structure (5, 6, 18). Such heterogeneity in the glycan structure and composition finely tunes the affinity of the Fc domain for binding to the different classes of type I and type II FcγRs (6, 14, 19)(Figure

1B). For example, the branching fucose residue regulates the Fc domain affinity specifically for the activating type I Fc γ R, Fc γ RIIIa, without any impact on the capacity of the Fc domain to interact with other classes of type I Fc γ Rs (20, 21). Based on the selectively enhanced binding affinity for Fc γ RIIIa, Fc glycoengineering has been successfully used as a strategy to generate antibodies with improved *in vivo* efficacy, as afucosylated IgG glycovariants exhibited enhanced Fc effector activity compared to their fucosylated counterparts (22–26).

In contrast, the presence of terminal sialic acid residues is associated with reduced binding to type I Fc γ Rs and preferential engagement of type II Fc γ Rs (18, 27, 28); an effect attributed to the induction of a conformational change of the CH2 domains upon sialylation that affects type I and type II Fc γ R binding (5, 8, 9). Indeed, sialylation of the Fc-associated glycan exposes a region at the CH2–CH3 interface, which serves as the binding site for type II Fc γ Rs (8, 9). This conformational change also results in the obstruction of the type I Fc γ R binding site at the hinge-proximal region of the CH2 domain, suggesting that Fc domain glycosylation modulates the capacity of the Fc domain to adopt two mutually exclusive conformations: an ‘open’ that enables for type I, but not type II Fc γ R binding, and a ‘closed’ that is induced upon sialylation and preferentially engages type II, but not type I Fc γ Rs (9).

Fc γ Rs: types, function and downstream signaling

The conformational state of the Fc domain regulates the interactions with two distinct types of Fc γ Rs: type I and type II that differ in terms of their structural domain organization as well as the stoichiometry by which they interact with the Fc domain (Figure 2A). Type I Fc γ Rs belong to the immunoglobulin (Ig) receptor superfamily and their extracellular, IgG binding region consists of two or three Ig-like domains (29, 30). Type I Fc γ Rs–Fc interactions are mediated through 1:1 binding of the Fc γ R loop region between the Ig-like domains with the hinge-proximal CH2 domain of the IgG Fc (31–33). In contrast, all type II Fc γ Rs are members of the C-type lectin receptors and recognize the Fc domain only at the closed conformation at a 2:1 (Fc:Fc γ R) stoichiometry through interactions with the CH2–CH3 domain interface that is exposed following acquisition of the closed conformation state induced upon sialylation (5, 8, 9).

In humans, type I Fc γ Rs are encoded by eight different genes, each with multiple transcriptional isoforms, located at a locus on the long arm of chromosome 1 (1q21–23). Genetic analysis suggested that the majority of the Fc γ R genes (with the exception of the genes encoding Fc γ RI) emerged throughout the evolutionary history through sequential gene duplication events of the ancestral Fc γ R gene locus, giving rise to the unique organization of the human IgG locus, which comprises several Fc γ R genes that share high degree of homology (34, 35). Type I Fc γ Rs are further sub-divided into high and low affinity receptors, based on their capacity to interact with monomeric or multimeric IgG, respectively. Fc γ RI represents the high affinity Fc γ R and is capable of interacting with monomeric IgG with relatively high affinity (K_d : 10^{-9} – 10^{-10} M). Three genes coding for Fc γ RI have been described: *FCGR1A*, *FCGR1B*, and *FCGR1C*; however, only *FCGR1A* encodes the prototypic high affinity receptor, Fc γ RI that is capable of high affinity IgG

binding (36). *FCGR1B* and *FCGR1C* possibly represent pseudogenes that arose from duplication of the *FCGR1A* gene and express truncated or soluble forms of Fc γ RI, with unknown or poorly characterized function (36, 37). In particular, the Fc γ RIb1 and Fc γ RIc transcripts contain premature stop codons within the extracellular domain of Fc γ RI, possibly representing soluble forms of the Fc γ RI receptor (36, 37). In contrast, the Fc γ RIb2 isoform is expressed as an intracellular protein, retained predominantly within the endoplasmic reticulum; however, its function remains unknown (37, 38). The increased affinity of Fc γ RI for IgG is attributed to its unique structure, as contrary to the low affinity Fc γ Rs (like Fc γ RII and Fc γ RIII), the α ligand-binding chain of Fc γ RI comprises three V-type, Ig-like domains that greatly stabilize Fc-Fc γ R interactions (39). The Fc γ RI α chain associates with a disulfide-bonded dimer of the Fc receptor γ chain (encoded by *FCER1G*), a signal transducing polypeptide initially described as a component of the high affinity IgE receptor Fc ϵ RI (40–42). Association of the Fc γ RI α chain with the FcR γ chain is necessary for receptor expression and signaling following receptor engagement (41, 43). Indeed, the FcR γ chain carries activating signaling motifs (ITAM – immunoreceptor tyrosine-based activation motif), which serves as the docking site for Syk family kinases and mediates signal transduction following IgG-Fc γ RI interactions (40, 44). Fc γ RI is constitutively expressed by monocytes and macrophages, as well as by many myeloid progenitor cells. Fc γ RI expression can also be induced in other myeloid cell types, like neutrophils and eosinophils, following stimulation with primarily interferon- γ (IFN- γ) and to a lesser extent by granulocyte-colony stimulating factor (G-CSF), interferon- α and interleukin-12 (45, 46)(Figure 2B).

Apart from the high affinity Fc γ RI, Fc γ Rs (like Fc γ RII and Fc γ RIII) display low affinity for monomeric IgG. Instead of engaging monomeric IgG, low affinity Fc γ Rs are only capable of binding to aggregated IgG through multiple multivalent, low affinity, high avidity interactions (33). This is particularly important, as low affinity Fc γ Rs can be engaged only by antibody-antigen complexes during an immune response, but not at steady state conditions by monomeric IgG in the absence of antigen. This ensures that engagement and crosslinking of low affinity Fc γ R occurs only during an immune response, preventing thereby inappropriate or excessive Fc γ R-mediated signaling at physiological conditions. All genes encoding the low affinity Fc γ Rs are clustered into a single Fc γ R locus, located at chromosome 1 and represent a series of multiple gene duplication and recombination events, followed by gain-of-function mutations of the ancestral Fc γ R locus, which can be traced back early in mammalian evolution as well as in marsupials (35). In humans, five low affinity Fc γ Rs have been described, along with several genetic variants (either in the form of non-synonymous substitutions and copy number variants) that impact Fc γ R expression and ligand binding affinity, influencing thereby disease susceptibility and severity for a number of chronic inflammatory and autoimmune pathologies (29). A common feature of all low affinity Fc γ Rs is the presence of two V-type, Ig-like domains that comprise the extracellular, ligand binding region (Figure 2A). A series of crystallographic, molecular modeling and mutational analyses have previously identified the ligand binding interface for each low affinity Fc γ R, indicating that the IgG binding region is localized in the second, membrane-proximal Ig-like domain, which is normally accessible for IgG interactions due to the horizontal orientation of the extracellular domain of the receptor (31–33, 47–50).

Despite the similarities between the low affinity Fc γ R in relation to their extracellular domains and binding interface, each Fc γ R exhibits unique characteristics in terms of their intracellular structure and signaling activities. Fc γ RIIa, Fc γ RIIb, and Fc γ RIIc (encoded by *FCGR2A*, *FCGR2B*, and *FCGR2C*, respectively) share a characteristic structure, which is the presence of functional signaling motifs in their intracellular domains. Fc γ RIIa, b, and c are expressed as a single chain (α chain) membrane receptors that comprised both the extracellular, IgG-binding region, as well as an intracellular signaling domain, without requiring association with the FcR γ chain for signaling or expression (30). The intracellular motifs of Fc γ RII include either an ITAM (immunoreceptor tyrosine-based activating motif) for Fc γ RIIa and Fc γ RIIc or an ITIM (immunoreceptor tyrosine-based inhibitory motif) for Fc γ RIIb (10). Fc γ RIIa is expressed by diverse cell types, including predominantly cells of myeloid origin, like polymorphonuclear leukocytes, macrophages, dendritic cells, monocytes, and platelets (10). Additionally, Fc γ RIIa expression has been reported in certain types of endothelial and epithelial cells, as well as in subsets of memory T cells (memory α/β T cells). Fc γ RIIb, the sole Fc γ R capable of transducing inhibitory signals upon crosslinking, exhibits two main splicing isoforms: b1 and b2. Fc γ RIIb1 represents the full-length transcript, which encodes a signal sequence that inhibits receptor internalization (51–54). In contrast, Fc γ RIIb2 is generated by skipping the exon that codes for this signal sequence, resulting in a variant that is capable of receptor internalization following IgG crosslinking (55). In addition to the differential internalization capacity, Fc γ RIIb1 and Fc γ RIIb2 also exhibit characteristic expression patterns. More specifically, Fc γ RIIb1, the full-length variant that is incapable for internalization, is predominantly expressed by cells of the lymphoid lineage, like B cells, whereas Fc γ RIIb2 expression is restricted to phagocytic, myeloid cells, like monocytes, neutrophils, macrophages, dendritic cells, and eosinophils (52)(Figure 2B). Fc γ RIIc is encoded by the *FCGR2C* gene and shares high degree of similarity with both *Fc γ RIIa* and *Fc γ RIIb*, probably reflecting a gene recombination event between *FCGR2A* and *FCGR2B* late in human evolution (35). Indeed, Fc γ RIIc represents a chimeric receptor comprising the extracellular, ligand binding region of Fc γ RIIb and the intracellular, ITAM-containing domain of Fc γ RIIa. Its expression is mainly restricted to NK cells (56); however, for the majority of human populations (70–90%), Fc γ RIIc expression is absent due to the presence of a premature stop codon at exon 3 (56, 57).

Similar to Fc γ RII receptors, Fc γ RIIIa and Fc γ RIIIb are characterized by relatively high sequence similarity, indicating a common origin for *FCGR3A* and *FCGR3B* genes, which encode Fc γ RIIIa and Fc γ RIIIb, respectively. Indeed, previous genetic analyses and comparison of the Fc γ R locus among the various primate species revealed that the *FCGR3B* gene emerged relatively late in evolutionary history as a result of gene duplication of *FCGR3A* followed by a point mutation at the extracellular, membrane-proximal region of the receptor (35, 58). This key point mutation created a glycosylphosphatidylinositol (GPI) anchor signal sequence, resulting in the processing of Fc γ RIIIb, as a GPI-anchored protein (59). This difference accounts for the distinct structural differences between Fc γ RIIIa and Fc γ RIIIb (Figure 2A). Whereas Fc γ RIIIa is a transmembrane protein that requires the association with the FcR γ chain for expression and signaling (44, 60), Fc γ RIIIb is post-translationally processed as GPI-anchored protein lacking intracellular signaling domains

(61). This difference also influences the receptor immunostimulatory activity following engagement. In contrast to Fc γ RIIIa, which is capable of transducing potent activating signals following receptor crosslinking through the FcR γ chain, Fc γ RIIIb lacks robust signaling capacity (59, 62). Fc γ RIIIb often relies on other receptors (like Fc γ RIIa) or accessory chains (like ζ chain or FcR γ chain) for signaling activity (42). The differences between Fc γ RIIIa and Fc γ RIIIb are not limited to their structure and signaling activity, but also extend to their expression pattern and distribution. Fc γ RIIIb expression is restricted to neutrophils, which constitutively express high levels of Fc γ RIIIb on their surface (61, 63). Other granulocyte subsets, like eosinophils also express Fc γ RIIIb, but only following induction with IFN- γ (5, 64). In contrast, Fc γ RIIIa expression is widely expressed by several leukocyte cell types, including macrophages, NK cells, and a subset of monocytes (CD14^{int}, CD16^{high}; patrolling monocytes) (5, 10, 65)(Figure 2B). Fc γ RIIIa expression levels are also greatly variable among the different tissue-resident macrophage and dendritic cell subsets (10).

Since almost all Fc γ Rs are capable of transducing intracellular signals following crosslinking by IgG complexes, Fc γ Rs are often divided into activating or inhibitory, based on their ability to transduce immunostimulatory or inhibitory signals (16). Common to all myeloid cell types is the concurrent expression of both activating and inhibitory Fc γ Rs on their surface with competing signaling activity. Differential engagement of activating or inhibitory Fc γ Rs could therefore influence the outcome of IgG-mediated inflammation; a concept that has been experimentally evaluated in several *in vivo* models of antibody-mediated cellular cytotoxicity (16, 64, 66, 67). Activating Fc γ Rs have the capacity to initiate a range of cellular activation processes through their activating signaling motifs (ITAMs), in processes resembling those described for other antigen receptors, like the T and B cell receptors (68). Most Fc receptors consists of heterodimers of the α subunit, which constitutes the ligand-binding domain, along with the FcR γ chain (either one or two) that contains the ITAM motifs necessary for signaling (40, 61, 69)(Figure 2A). FcR γ chain (encoded by *FCER1G*) is structurally and functionally related to the ζ chain of the T cell receptor and are both located at the same locus on chromosome 1. FcR γ chain normally forms γ - γ homodimers that associate with the α subunit through transmembrane domain interactions of an aspartic acid residue of the γ chain with basic residues of the α subunit (51, 70). Apart from γ - γ homodimers, heterodimers between the FcR γ chain and ζ chain have been reported to associate with the FcR α subunit *in vitro*; however, it is unclear whether this association occurs naturally (71).

In contrast to the Fc γ Rs that require the association with the FcR γ chain for expression and signaling, Fc γ RIIa and Fc γ RIIc comprise only a single α subunit that not only has the capacity to interact with the Fc domain of IgG, but also possesses an ITAM signaling motif within its intracellular domain. These receptors, which are uniquely found in humans, have the capacity to transduce activating signals following receptor crosslinking, in a process similar to Fc γ Rs that require association with the FcR γ chain, like Fc γ RIIIa. Several biochemical and biophysical studies have elucidated the precise signaling pathways that are activated following binding of IgG complexes to activating Fc γ Rs (10, 72). Since signaling of activating Fc γ Rs is mediated through motifs (ITAM) or accessory signaling proteins (like the FcR γ chain) that are also found in the high affinity IgE receptor, Fc ϵ RI, both Fc γ and

Fcε receptors share a number of common features related to their signaling activity following receptor crosslinking. Indeed, initial studies on the high affinity FcεRI receptor have provided useful insights into the mechanisms by which activating FcγRs transduce intracellular signals (73–75). Apart from the difference in their capacity to interact with monomeric IgGs, high and low affinity FcγRs also differ in relation to the sequence of events that are required for the initiation of downstream signaling. For example, high affinity receptors require binding of monomeric antibodies that are subsequently aggregated through the recognition and binding of multivalent antigens to their Fab domains (73, 75). In contrast, low affinity FcγRs can only engage antibody-antigen complexes, but not monomeric antibodies. Binding of such complexes is accomplished through multiple low affinity, high avidity interactions that facilitate receptor clustering and subsequent initiation of intrinsic signals (76, 77). Despite the differences between high and low affinity FcγRs in the events preceding receptor crosslinking, all activating FcγRs share a common pattern of signal cascade activation following crosslinking. In particular, upon receptor aggregation, two or more ITAM domains present in the FcR γ chain or in the intracellular region of the α subunit of FcγRIIa and FcγRIIc become cross-phosphorylated by Src and Syk family kinases, including Lyn, Lck, Hck, and Fgr (62, 78–83). In addition to these kinases, phosphorylation of additional proteins including FAK, ZAP-70 and ζ chain subunits have been reported following receptor aggregation (81, 83, 84). Initial activation of Syk and Src family kinases is accompanied by subsequent activation of downstream signaling pathways, including the activation of phospholipase C γ (PLCγ), which in turn generates metabolites that lead to the activation of protein kinase C (PKC) and inositol triphosphate (IP³), triggering a rapid increase in intracellular Ca²⁺ levels through mobilization of the endoplasmic reticulum Ca²⁺ pool (85, 86). Elevated intracellular Ca²⁺ levels also activate a number of Ca²⁺-regulated signaling proteins, including PKC (87, 88). Finally, a number of late signaling pathways become activated following FcγR crosslinking, including the Ras pathways, kinases of the MEK and MAP family, as well as transcriptional induction of several cytokine and chemokine genes along with genes involved in cell survival, differentiation, and motility through the activation of transcription factors, such as NF-κB and NFAT (85, 89, 90).

Contrary to the activating, ITAM-bearing FcγRs, two FcγRs, FcγRIIIb and FcγRIIb, have been described that either lack intrinsic signaling capacity or initiate immunosuppressive signals, respectively. As mentioned above, FcγRIIIb is uniquely found in humans and exhibits structural features characterized by the absence of intracellular domains necessary for signaling. Although FcγRIIIb is incapable of transducing intracellular signals following crosslinking by IgG complexes, FcγRIIIb-Fc interactions have been previously shown to result in the induction of cellular activation, mainly through the synergistic activity of FcγRIIIb with other receptors, like FcγRIIa and complement receptors, expressed on the surface of neutrophils (63, 91).

FcγRIIb represents the sole FcγR with inhibitory activity that has the capacity to transduce intracellular signals that directly antagonize the immunostimulatory signals of activating FcγRs. In contrast to the other FcγRII receptors, FcγRIIb comprises an ITIM domain at its intracellular region that mediates inhibitory cellular signaling. Several studies, mainly on B cells, have previously dissected the exact molecular mechanisms of FcγRIIb-mediated

cellular inhibition. In particular, co-aggregation of Fc γ RIIb with activating receptors, like the B cell receptor or activating Fc γ Rs, results in phosphorylation of Fc γ RIIb ITIM domains, which in turn recruits SHIP and SHP2 phosphatases (68, 92, 93). In the case of Fc γ RIIb-activating Fc γ R crosslinking, recruitment of these phosphatases to the Fc γ RIIb ITIM domains prevent phosphorylation of Syk and Src family kinases, counterbalancing thereby any activating signals originating from activating Fc γ Rs (94, 95). Similarly, on B cells, recruited SHIP and SHP2 phosphatases interfere with B cell receptor signaling by hydrolyzing phosphoinositide intermediates, such as phosphatidylinositol-3,4,5-triphosphate, preventing thereby the recruitment of PH-domain containing kinases, like PLC γ and BTK, and the activation of downstream signaling pathways (68, 93). Since Fc γ RIIb is the only Fc γ R with inhibitory activity, it plays a central role in regulating Fc γ R-mediated inflammation and B cell receptor activity. Indeed, Fc γ RIIb regulates key processes in B cells, related to cell activation, selection, and antibody production (95, 96), as well as in myeloid cells, like macrophages, influencing the outcome of IgG-mediated inflammation. Several examples from *Fcgr2b* knock-out mice exist that highlight the importance of Fc γ RIIb in regulating B cell activation, antibody affinity selection, and antibody serum levels (96, 97). Likewise, genetic deletion of *Fcgr2b* results in enhanced pro-inflammatory activity in macrophages in murine models of immune complex-mediated alveolitis and collagen-induced arthritis (66, 98–100). Additionally, studies in humans revealed that polymorphisms in the promoter region or transmembrane domain of Fc γ RIIb that influence receptor expression or activity, respectively are associated with susceptibility to autoimmune disorders, further highlighting the significance of Fc γ RIIb in maintaining peripheral tolerance and regulate IgG-mediated inflammatory processes (29, 101–104).

As mentioned above, type II Fc γ Rs belong to the C-type lectin family of receptors that exhibit ligand binding specificity exclusively for the “closed” conformation of the IgG Fc domain (5, 8, 9). Two main type II Fc γ Rs have been identified so far: DC-SIGN and CD23 with distinct biological activity *in vivo* (8, 9, 28, 105)(Figure 2A). Although sialylated IgG Fc binding specificity has been recently shown for other C-type lectin receptors, such as CD22 and DCIR, their precise biological significance has not been fully investigated (106–108). DC-SIGN is encoded by the *CD209* genes, which is mapped at the same locus with the genes that codes for CD23 (*FCER2*), indicative of a common functional and structural origin. Indeed, both CD23 and DC-SIGN are both heavily glycosylated membrane proteins that belong to the C-type lectin receptor superfamily. Since the capacity of type II Fc γ Rs to engage IgG has been recently described, the precise signaling pathways have not been defined as extensively as for type I receptors. However, a number of recent studies have elucidated the mechanisms that regulate IgG binding to type II Fc γ Rs, as well as the downstream biological consequences following receptor engagement (9, 27, 105, 109, 110).

DC-SIGN has the capacity to engage diverse carbohydrate ligands and heavily-glycosylated glycoproteins, including mannose-rich glycan structures, and pathogen surface glycoproteins, such as gp160, the HIV-1 envelope glycoprotein (28, 111). Binding of these ligands to DC-SIGN is accomplished predominantly through carbohydrate-mediated interactions. In contrast, sialylated IgG Fc – DC-SIGN binding is mediated through protein interactions with the amino acid backbone of the Fc domain at the CH2–CH3 interface; a region that becomes exposed following conformational alterations induced by the Fc-

associated glycan structure (8, 9, 28). Since DC-SIGN has the capacity to interact with diverse ligands, downstream signaling cascades following receptor engagement depend largely on the nature of the ligand. In the case of DC-SIGN engagement by sialylated IgG Fc, expression of interleukin 33 (IL-33) is induced following receptor engagement on regulatory macrophages. This effect triggers a robust Th-2 polarizing response that induces potent Treg responses, effectively suppressing Th1- and Th17-mediated inflammation (109, 110). Likewise, IL-33 triggers the production and release of IL-4 by basophils that results in Fc γ RIIb upregulation on effector myeloid cells, including monocytes and macrophages, at sites of inflammation (109).

CD23 was initially described as a low affinity receptor for IgE; however, recent studies showed that CD23 exhibits ligand binding activity for sialylated IgG in addition to IgE (9, 105). Molecular modeling of the CD23:IgE interaction and the potential CD23:sialylated IgG Fc complex first suggested that CD23 might have the capacity for binding to both ligands (9). IgE interactions with CD23 are largely attributed to the intrinsic flexibility of the C ϵ 3 domain (functionally related to the CH2 domain of IgG). In an analogous model, sialylation of the Fc-associated glycan increases the flexibility of the C γ 2 domain, thereby conferring flexibility to the Fc and enabling Fc interactions with CD23 (9, 112, 113). In terms of expression, CD23 exhibits two splice variants: CD23a and CD23b, which differ both in relation to their expression pattern and responsiveness to IL-4 (114). CD23a is constitutively expressed by B cells, whereas CD23b expression is induced only following IL-4 treatment and is limited to myeloid cells, such as monocytes, macrophages and granulocytes, as well as in certain T cell subsets (114). Apart from sialylated IgG, CD23 can also interact with many other ligands, including CD21, CD11c, and CD11b; however, the functional consequences of these interactions have not been characterized (115).

Cellular effects of Fc γ R signaling on myeloid cells

Fc γ R engagement and the associated signaling pathways that are activated upon receptor crosslinking modulate the function of diverse immunoregulatory pathways that shape immune responses, regulate cellular activation and modulate IgG-mediated inflammation (10)(Figure 3). B cells, a lymphoid cell type expressing only one class of each Fc γ R type (the type I Fc γ RIIb and the type II CD23) represent a bona fide example of complex Fc γ R-mediated signaling cellular regulation. In particular, CD23-mediated signaling on B cells following engagement with sialylated IgG complexes modulates the expression of Fc γ RIIb in these cells (105). Increased Fc γ RIIb expression on B cells drives the generation of high affinity antibody responses, through the regulation of B cell receptor signaling and modulation of B cell selection (105). Likewise, Fc γ RIIb on antibody-producing plasma cells regulate cell survival and antibody expression; a key process during the resolution phase of the humoral immune response (93, 97).

In contrast to lymphoid cells, cells of the myeloid lineage, including granulocytes, macrophages, and dendritic cells, co-express several classes of Fc γ Rs on their surface during all stages of their differentiation. It is therefore anticipated that Fc γ R engagement in these cells could have diverse immunomodulatory consequences that regulate key processes during an immune response, affecting the outcome of IgG-mediated signaling. Indeed, Fc-

Fc γ R interactions on myeloid cells mediate diverse effector functions including cellular activation, cytotoxicity, and phagocytosis of IgG-opsonized targets (5, 10). Additionally, Fc γ R-mediated uptake of IgG complexes by phagocytes influences antigen processing and presentation, and regulates the differentiation and maturation of antigen presenting cells, modulating thereby T cell responses (10, 67). Since downstream Fc γ R signaling leads to the transcriptional activation of several cytokine and chemokine genes, diverse effects from such responses impact cell mobilization, migration, differentiation, and survival. In the next sections, the cellular and biological effects of Fc γ R signaling initiated upon interactions with the Fc domain of IgG are discussed in detail, focusing on the significance of Fc γ R pathways in the modulation of myeloid cell functional activity (Figure 3).

Cell Activation

Engagement of ITAM-bearing type I Fc γ Rs by IgG complexes initiates a number of signaling cascades that lead to cellular activation and subsequent induction of effector functions. Cellular responses to Fc-Fc γ R interactions vary between myeloid cell types; however, Fc γ R aggregation typically leads to rapid internalization of Fc γ Rs and activation of different signaling pathways that influence cell activation (61, 68, 69, 116). Among myeloid cell types, activation induced upon Fc γ R engagement is most profound in granulocytes and platelets, as these cell types rapidly mediate biological effects within a few minutes following stimulation with IgG complexes (61, 63, 76, 117, 118). Granulocytes represent the most abundant leukocyte cell type in circulation and mediate pleiotropic effector functions during an inflammatory response to invading pathogens (119). Indeed, granulocytes, which include neutrophils, eosinophils, and basophils, are the first leukocyte cell type recruited to sites of inflammation in response to infection or injury and play a central role in immunity against bacterial, viral, and fungal pathogens (120). Several studies have examined the role of Fc γ R-mediated cellular activation and determined the downstream functional consequences upon Fc γ R engagement of granulocytes. Granulocyte effector responses initiated following cellular activation in response to Fc-Fc γ R interactions aim at the destruction of invading pathogens, and involve the release of an array of microbicidal molecules. These molecules are either preformed and stored in specialized granules in the cytoplasm or are rapidly de novo generated (120). Activation of Syk and Src family kinases upon Fc γ R crosslinking triggers the generation of reactive oxygen intermediates (ROI) through the formation and activation of the NADPH-dependent oxidase complex; a multi-component protein complex that consists of p40^{phox}, p47^{phox}, p67^{phox}, p22^{phox}, and gp91^{phox} (121–124). Phosphorylation of p47^{phox} promotes its physical association with cytochrome b558, triggering the rapid generation of superoxide anions. Superoxide anions can have direct cytotoxic activity or can lead to the generation of reactive oxygen or nitrogen intermediates, including hydroxyl radical (OH $\cdot$), hypochlorous acid (HOCl), and peroxynitrite (ONOO $^-$) (122, 125, 126). Additionally, superoxide anions can be dismutated by superoxide dismutase, leading to generation of hydrogen peroxide (127). The release of antimicrobial molecules by granulocytes in response to FcR crosslinking represents one of the most powerful immune mechanisms in the human body. Apart from these chemical compounds, activation of the PKC pathway and increase in the intracellular Ca²⁺ concentration following Fc γ R-mediated cellular activation, triggers the mobilization and release of pre-formed molecules stored in specialized granules (128). The content of

these cytoplasmic granules varies among the different granulocyte subtypes; however, it typically comprises proteases (elastase, cathepsins, and collagenases), antimicrobial peptides and proteins (lysozyme, defensins, lactoferrin), enzymes (peroxidase, alkaline phosphatase), lipid mediators (leukotrienes), as well as cell surface receptors (CD11b) (129–135). Release of these mediators at inflammatory sites represents a hallmark for granulocyte activation and constitutes an important effector mechanism by which granulocytes mediate *in vivo* activity against invading pathogens.

In an analogy to granulocyte activation and degranulation, Fc γ R-mediated crosslinking also has the capacity to induce platelet activation. Human platelets express Fc γ RIIa, an activating type I Fc γ R, which, upon crosslinking, mediates potent signaling activity (136–138). Additionally, they express FcR γ chain, an important accessory signaling subunit for platelet function that associates with several platelet surface receptors, including GPVI (139, 140). Activation of downstream signaling pathways trigger platelet activation and degranulation in a process similar to that observed for granulocytes (141). Elevation of the intracellular Ca²⁺ concentration following Fc γ R crosslinking triggers rapid degranulation and release of platelet granule content (139, 142). This includes cell surface receptors that participate in cell-cell adhesion and platelet aggregation, as well as molecules involved in the activation of fibrin cascade, including fibronectin, fibrinogen, and coagulation factors V and XIII (139). Additionally, platelet degranulation is associated with the release of pro-inflammatory cytokines and chemokines, including IL-1 β , and IL-8, as well as growth factors and pro-survival factors that influence leukocyte cell survival, differentiation, and effector activity (143–145). These events highlight the potential of Fc γ R-mediated signaling to trigger platelet activation and thrombogenesis, as well as to influence leukocyte function.

Phagocytosis and Antigen Presentation

A common function of all type I Fc γ Rs is their capacity to mediate efficient phagocytosis of IgG-opsonized particles, which can range from small antigens (toxins, infectious pathogens, etc.), to whole cells. Although the phagocytic capacity varies greatly among different myeloid phagocytes, the downstream signaling events and mechanisms that characterize Fc γ R-mediated phagocytosis follow the general pattern of Fc γ R crosslinking and cellular activation. In particular, Fc γ R crosslinking by immune complexes triggers receptor internalization, and activation of downstream signaling pathways that facilitate actin remodeling and endosomal uptake and sorting (10, 63, 77, 83, 146). Although in myeloid cells both activating and inhibitory Fc γ Rs are capable of internalization and phagocytosis, uptake through activating Fc γ Rs mediates more potent effector responses associated with the induction of pro-inflammatory signaling pathways (69, 83, 147). Activation of Syk family kinases upon receptor crosslinking mediates cellular activation, thereby influencing leukocyte effector function. Indeed, activating Fc γ R-mediated phagocytosis is associated with enhanced endosomal maturation and lysosomal fusion, facilitating antigen processing and presentation on MHC II molecules (69, 148–150). These effects result in the induction of more robust T cell response, further augmenting the *in vivo* protective activity of antibodies (66, 98, 151, 152).

Several studies have demonstrated the key role of Fc-Fc γ R interactions in inducing clearance of IgG-coated particles *in vivo*. For example, seminal experiments using IgG-opsonized erythrocytes demonstrated that their uptake is mediated exclusively through Fc γ R-mediated pathways by splenic macrophages (153, 154). Likewise, the *in vivo* activity of antibodies against toxins and bacterial pathogens depends on interactions with Fc γ Rs expressed by effector leukocytes; experimentally, this can be shown using mice that are genetically modified to lack expression of specific Fc γ Rs required for IgG-mediated protection or through modification of the Fc domain on protective antibodies to abrogate Fc γ R interactions, thus greatly reducing their *in vivo* activity (58, 155–162). Similarly, clearance of tumors or infected cells targeted by antibodies against surface antigens requires interactions with activating Fc γ Rs (16, 66, 67, 154, 156, 163–167).

Dendritic Cell Maturation

Under homeostatic conditions, human dendritic cells express two type I Fc γ Rs, the inhibitory Fc γ RIIb and the activating Fc γ RIIa (5). These receptors are co-expressed on the surface of dendritic cells and activating signals from Fc γ RIIa engagement are counterbalanced by the inhibitory activity of Fc γ RIIb, which prevents undesired DC maturation and differentiation. The balance of activating and inhibitory signaling is a key regulatory process controlling DC activity; therefore, the level of expression of these two Fc γ Rs must be tightly regulated. Inflammatory microenvironments can trigger expression of additional activating type I Fc γ Rs, such as Fc γ RI and Fc γ RIIIa, as well as influence the expression of Fc γ RIIb on DCs. For example, IL-4 has been previously shown to induce Fc γ RIIb upregulation in dendritic cells, whereas IFN γ decreases Fc γ RIIb expression and stimulates Fc γ RI expression (46, 109, 151, 168, 169). This finely-tuned balance between activating and inhibitory Fc γ Rs expression and activity determines the threshold for immune complex-mediated dendritic cell activation and responsiveness to PAMPs. Indeed, stimulation of dendritic cells with IgG immune complexes often does not induce robust cell maturation, but requires co-stimulatory signals, such as TLR signaling to overcome the inhibitory activity of Fc γ RIIb (67, 98, 116, 151, 152, 168, 170). Skewing the balance of the contrasting signaling activity of dendritic cell Fc γ RIIa and Fc γ RIIb has profound consequences in cell maturation and the development of subsequent T cell responses. Indeed, genetic deletion or antibody-mediated block of Fc γ RIIb ligand binding activity on dendritic cells greatly augments immune complex-mediated cell maturation, resulting in the upregulation of MHC and co-stimulatory molecules, as well as in enhanced antigen presentation and T cell activation (116, 151, 152, 168, 171). Likewise, in a model of CD20⁺ lymphoma, preferential engagement of dendritic cell Fc γ RIIa through Fc domain engineering of anti-CD20 antibodies, resulted in improved antigen-specific T cell responses; an effect attributed to an increase in the threshold for Fc γ RIIb-mediated inhibition of dendritic cell maturation (67). These studies highlight the importance of the balancing activity of activating and inhibitory Fc γ Rs in regulating dendritic cell activation, thereby influencing adaptive immune responses.

Macrophage Polarization

Human macrophage populations represent a continuum of diverse activation states with distinct functional and phenotypic characteristics (172). Macrophage polarization has been

originally divided into two broad phenotypes – M1 and M2, induced by the contrasting activity of Th1, like IFN- γ and Th2 cytokines, like IL-4, respectively (173). However, it was soon appreciated that immune complex-mediated effector pathways represent an additional determinant for macrophage polarization. In monocytes and polarized macrophages, signaling through the activating Fc γ R_s is associated with the upregulation of several pro-inflammatory cytokines and chemokines (10, 58). However, when activating Fc γ R signaling is coupled with stimulation through TLRs, like TLR4 in non-polarized macrophages, this synergistic signaling activity triggers induction of a specific polarization state that resembles the M2 phenotype (174–176). This phenotype, which is generally termed as M2b or “regulatory”, is characterized by increased IL-10, IL-1 and IL-6 expression as well as by increased migratory and phagocytic capacity (174–176). A number of *in vivo* studies utilizing mouse strains with genetic deletion of *Fcgr2b* have provided useful insights into the contribution of Fc γ R-mediated signaling in regulating macrophage polarization and functional activity. For example, Fc γ R_{IIb} deficient mice exhibit lower macrophage activation threshold upon challenge with immune complexes and present a more severe phenotype in models of immune complex-induced shock, arthritis, and alveolitis. (100, 153, 174). Also, Fc γ R_{IIb} expression can determine susceptibility to infection; for example, *Fcgr2b*^{-/-} mice exhibit improved bacterial clearance in models of pneumococcal peritonitis (177, 178), whereas, overexpression of Fc γ R_{IIb} is associated with increased mortality upon challenge with *Streptococcus pneumoniae* (177, 178). These findings highlight the importance of Fc γ R-mediated signaling in macrophage polarization and in the regulation of macrophage effector function *in vivo*.

Fc receptors in disease

Balanced signaling through Fc receptors is required for proper immune activity and health; indeed, Fc γ R polymorphisms that affect signaling are associated with a variety of human diseases. In particular, specific autoimmune diseases are more commonly found in association with Fc γ R polymorphisms that confer lower affinity Fc-Fc γ R interactions. For example, the R131 variant of Fc γ R_{IIa} has lower affinity for the IgG2 Fc domain when compared with the H131 form and is more commonly found in people with systemic lupus erythematosus (SLE)(179), antiphospholipid syndrome (180), myasthenia gravis (181), and severe Guillain-Barre syndrome (182). The frequency of individuals homozygous for either the R131 or H131 variant of Fc γ R_{IIa} varies between ~25–35% depending on ethnicity (183). In addition, polymorphisms that reduce transcription or disrupt membrane localization of the inhibitory Type I FcR, Fc γ R_{IIb} are associated with SLE in humans (102–104, 184). The role of Fc γ R_{IIb} in maintenance of immune tolerance and production of high affinity antibody responses has been studied in some detail; Fc γ R_{IIb} signaling on B cells increases the threshold for the affinity of B cell receptor that is required for cell survival, thus low Fc γ R_{IIb} expression or signaling can result in autoantibody and low-affinity antibody production (96, 97, 105). Many Fc γ R variants have been described and are reviewed elsewhere (29, 185). Studies that provide clear mechanistic evidence for the role of specific Fc γ R_s in health and disease are often performed in animal models. Here, we briefly review works demonstrating the role of specific Fc γ R_s in infection, immunosuppression and

cytotoxicity along with clinical studies on Fc γ R polymorphisms that may support the basic findings.

Immunity to infectious pathogens

Type I Fc γ Rs contribute to protection against a variety of infectious pathogens *in vivo*. This is exemplified by experiments demonstrating that antibodies specific for influenza virus proteins, which do not exhibit neutralizing activity *in vitro*, can have protective activity *in vivo* that depends on activating Type I Fc γ Rs (157, 158). Similarly, suppression of simian-human immunodeficiency virus viremia by anti-HIV mAbs in macaques was dependent on Fc interactions with activating Fc γ Rs (156). Numerous studies have implicated Fc γ Rs in antibody-mediated protection against bacterial, viral and fungal pathogens (58). Clinical studies have found a correlation between the low affinity variant of Fc γ RIIa (R131) with increased susceptibility to severe bacterial infections and sepsis (186–188), suggesting a protective role for myeloid cell Fc γ RIIa-mediated effector functions in protection from infection in humans.

Disease enhancing IgG

A long-appreciated yet not fully understood immunomodulatory property of type I Fc γ Rs is their occasional ability to mediate enhanced infectious disease. An example of this phenomenon can be observed in secondary infection with dengue virus; while primary infection is often asymptomatic or mild in presentation, subsequent infection with a distinct dengue serotype can be associated with enhanced viral replication and disease (189, 190). This enhanced secondary disease is, at least in part, thought to be mediated by cross-reactive, non-neutralizing IgGs generated during the primary virus exposure that enhance infection of Type I Fc γ R bearing cells, including monocytes, macrophages and dendritic cells. Antibody-dependent enhancement in dengue virus (DENV) infection has been demonstrated in a variety of *in vitro* and *in vivo* DENV infection models, but the mechanisms underlying enhancement are not well understood (191–194). Studies have shown that Fc γ R-mediated internalization of dengue viruses can result in more infected cells (195, 196), enhanced viral fusion activity (197) and suppression of innate immune signaling (198, 199). Severe DENV disease has been associated with specific combinations of virus serotypes and preexisting serotype immunity (200), viral genetic factors (201–204), and several host factors (205–209).

Antibody-dependent enhancement of disease that is not secondary to increased microbial replication has been observed during respiratory syncytial virus outbreaks in patients previously vaccinated with formalin-inactivated viral proteins and in some severe influenza virus infections. In these circumstances, immune complexes formed from non-neutralizing IgGs with viral proteins are thought to have mediated cytotoxicity and/or complement deposition and inflammation (210, 211). Why some microbes can cause increased infectivity and/or clinical disease through antibody-mediated mechanisms is not well understood. Adaptation to productive replication in monocytes or macrophages may be one determinant of cytokine-associated disease enhancement. A second determinant might be antigenic variability of a pathogen, which often results in production of cross-reactive, non-neutralizing antibodies that can enhance pathogen uptake by Fc γ R-expressing cells or, in

rare circumstances, may form insoluble immune complexes that cause disease due to type I Fc γ R-mediated inflammation or direct cytotoxicity.

Immunosuppressive IgG

A prime example of immunosuppressive activity through Fc γ Rs is the CD209/DC-SIGN-mediated anti-inflammatory activity achieved clinically through administration of high-dose intravenous immunoglobulin (IVIG) (28). This activity is dependent on the presence of IgGs modified by glycans with α 2,6-linked terminal sialic acid, which induce conformational flexibility of the Fc CH2 domain, enabling binding to Type II receptors. A mechanism underlying the anti-inflammatory signaling induced by IVIG has been described whereby sialylated Fc domains interact with DC-SIGN on regulatory myeloid cells, triggering IL-33 production, which, in turn, induces expansion of IL-4-producing basophils that promote increased expression of the inhibitory Fc receptor Fc γ RIIb on effector macrophages (109).

Cytotoxic IgG and immunity to tumors

Passively administered anti-tumor mAbs are key therapeutics in a number of cancers and often mediate their cytotoxic effects through mechanisms that depend on Fc γ R expression on effector myeloid cells (66, 212). Fc γ RIIIa expression on macrophages has been shown to mediate cytotoxicity of an anti-CD20 mAb in mice that were humanized for Fc receptor expression (67). Several clinical studies support the dependence of cytotoxic mAb activity on Fc γ RIIIa. Most prominently, the high binding variant of Fc γ RIIIa, V158, which confers up to ten-fold higher binding to IgG1 and significantly enhanced ADCC activity, has been associated with improved survival in several studies of cancer patients administered antibody therapeutics. Significantly increased response rates and survival are documented in lymphoma patients treated with Rituximab who were heterozygous or homozygous for the Fc γ RIIIa V158 polymorphism, for example. (213–215). In addition to direct cytotoxicity, long-term anti-tumor immunity has been demonstrated in some patients after administration of Rituximab (216). In mice, induction of anti-tumor immunity by an IgG1 anti-CD20 mAb could be generated by a mechanism dependent on Fc γ RIIIa, expressed on dendritic cells in humans (67).

Conclusions

IgG antibodies recruit effector cells through engagement of Type I and Type II Fc γ Rs. In this way, Fc-Fc γ R interactions represent a central tie between the humoral and cellular immune divisions. As outlined briefly here, these interactions are essential for immune mechanisms that can protect against infectious organisms and tumors and can, on occasion, mediate enhanced disease. Antibody therapeutics for the treatment of inflammatory or infectious diseases or cancers require specific effector functions and their development must consider not only target specificity but also downstream effector functions that will be required for optimal therapeutic efficacy.

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Figure 1. Structure and composition of the Fc-associated N-linked glycan regulates binding to type I and type II FcγRs

(A) An N-linked glycan structure is attached at the CH2 domain of each of the two heavy chains of IgG and consists of a core heptasaccharide structure that is composed of fucose, galactose, N-acetylglucosamine and sialic acid residues. (B) The structure and composition of this Fc-associated glycan determine binding specificity and affinity of the IgG Fc domain for different types of FcγRs. Several glycoform combinations exist with distinct binding capacity to interact with type I and type II FcγRs.

Figure 2. Overview of type I and type II FcγR structure and expression pattern in myeloid cell populations

(A) Structural characteristics of type I and type II FcγRs. Type I FcγRs belong to the immunoglobulin receptor superfamily and are composed of two or three extracellular Ig-like domains that interact with the IgG Fc domain at the hinge proximal region of the CH2 domain. Type II FcγRs comprise DC-SIGN and CD23, both C-type lectin receptors, that bind sialylated Fc IgGs. (B) Expression pattern of type I and type II FcγRs in myeloid leukocyte populations. The expression of several FcγRs is determined by cell differentiation status and is regulated by cytokines, like IL-4 and IFNγ.

Granulocytes	Cell activation	Macrophages	Macrophage polarization
	ROS production		Cell survival & differentiation
	Degranulation		Phagocytosis
	Phagocytosis		Antigen processing & presentation
	Chemokine & cytokine expression		Chemokine & cytokine expression
Platelets	Degranulation	Dendritic Cells	Dendritic cell maturation
	Aggregation		Upregulation of co-stimulatory molecules
	Thrombogenesis		Antigen uptake & presentation
Monocytes	Cell activation		Enhanced T cell activation
	Phagocytosis		Cytokine & chemokine responses
	Cell survival		
	Chemokine & cytokine expression		

Figure 3. Effector functions and processes that are regulated by Fc-FcγR interactions

Engagement of type I and type II FcγRs by the Fc domain of IgG initiates signaling cascades with diverse pro-inflammatory, anti-inflammatory and immunomodulatory consequences on myeloid cells, including granulocytes, monocytes, macrophages, dendritic cells and platelets.